AL/OE-TR-1994-0144
WRAIR-TR-1994-0016

ARMSTRONG LABORATORY

REPRODUCTIVE TOXICITY SCREEN OF 1,3,5-TRINITROBENZENE ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

E. R. Kinkead
R. E. Wolfe
C. D. Flemming

MANTECH ENVIRONMENTAL
TECHNOLOGY, INC.
P.O. BOX 31009
DAYTON, OH 45437

D. J. Caldwell
C. R. Miller

US ARMY MEDICAL RESEARCH DETACHMENT
WALTER REED ARMY INSTITUTE OF RESEARCH

G. B. Marit

OCCUPATIONAL AND ENVIRONMENTAL HEALTH DIRECTORATE
TOXICOLOGY DIVISION, ARMSTRONG LABORATORY
WRIGHT-PATTERSON AFB, OH 45433-7400

October 1994

FINAL REPORT FOR THE PERIOD SEPTEMBER 1993 THROUGH JUNE 1994

AIR FORCE MATERIEL COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

NOTICES

When US Government drawings, specifications or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from the Armstrong Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with the Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22314

DISCLAIMER

This Technical Report is published as received and has not been edited by the Technical Editing Staff of the Armstrong Laboratory.

TECHNICAL REVIEW AND APPROVAL

AL/OE-TR-1994-0144
WRAIR-TR-1994-0016

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1994	Final - September 1993 - June 1994

4. TITLE AND SUBTITLE
Reproductive Toxicity Screen of 1,3,5-Trinitrobenzene Administered in the Diet of Sprague-Dawley Rats

5. FUNDING NUMBERS
Contract F33615-90-C-0532
PE 62202F
PR 6302
TA 630200
WU 63020002

6. AUTHOR(S)
E.R. Kinkead, R.E. Wolfe, C.D. Flemming, D.J. Caldwell, C.R. Miller, and G.B. Marit

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
ManTech Environmental Technology, Inc.
P.O. Box 31009
Dayton, OH 45437-0009

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Armstrong Laboratory, Occupational and Environmental Health Directorate
Toxicology Division, Human Systems Center
Air Force Materiel Command
Wright-Patterson AFB OH 45433-7400

10. SPONSORING;MONITORING AGENCY REPORT NUMBER
AL/OE-TR-1994-0144
WRAIR-TR-1994-0016

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Several Army installations targeted for restoration have measurable quantities of 1,3,5-trinitrobenzene (TNB) in the soil and ground water. As part of the process to develop environmental and health effects criteria for restoration, a modified Screening Information Data Set (SIDS) reproductive study was performed. Male and female Sprague-Dawley rats received diet containing approximately 300, 150, or 30 mg TNB/kg diet. Mating occurred following 14 days of treatment. All dams, one-half the males, and representative pups were maintained for a total of 90 days of treatment. No mortality occurred during the study; however, a decrease in mean body weights was noted in both sexes of high-dose rats. A dose-related effect was noted in measurements of sperm function/activity. Sperm depletion and degeneration of the seminiferous tubules were noted histopathologically. Methemoglobinemia and splenic hemosiderosis were common findings in the high- and mid-dose levels of both sexes at necropsy. No adverse effects were noted in mating or fertility indices. No significant treatment-related differences were found in length of gestation, sex ratio, gestation index, or mean number of pups per litter.

14. SUBJECT TERMS

1,3,5-trinitrobenzene	Dietary	Explosives	Oral
Methemoglobinemia	Reproduction	Reproductive Screen	Nitrate
Seminiferous	Tubule Degeneration	Sprague-Dawley Rat	

15. NUMBER OF PAGES
50

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

THIS PAGE INTENTIONALLY LEFT BLANK

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit, ManTech Environmental Technology, Inc. This document serves as a final report on the reproductive toxicity screen of 1,3,5-trinitrobenzene administered in the diet of Sprague-Dawley rats. The research described in this report began in September 1993 and was completed in June 1994 under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. A02). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division. This study was sponsored by the U.S. Army under the direction of LTC Daniel J. Caldwell, USAMRD/WRAIR.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors gratefully acknowledge the technical assistance of Richard J. Godfrey, Willie J. Malcomb, TSgt Lynda M. Maynard, Jerry W. Nicholson, Stephanie A. Salins, SSG Brett W. Collier, Maj Donald R. Tocco, and Merry J. Walsh.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

dL	Deciliter
DNB	1,3-Dinitrobenzene
fL	Femtoliter
g	Gram
h	Hour
HCT	Hematocrit
HGB	Hemoglobin
kg	Kilogram
L	Liter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MetHb	Methemoglobin
mg	Milligram
mL	Milliliter
mm	Millimeter
mmol	Millimole
N	Number
p	Probability
pg	Picogram
RBC	Red blood cells
SEM	Standard error of the mean
TNB	1,3,5-Trinitrobenzene
TNT	2,4,6-Trinitrotoluene

SECTION 1

INTRODUCTION

Several Army installations targeted for restoration have measurable quantities of 1,3,5-trinitrobenzene (TNB) in the soil and groundwater. 1,3,5-Trinitrobenzene is a dimorphic crystalline solid that is easily dissolved in organic solvents (Fedoroff et al., 1962). It is produced during the nitration step of trinitrotoluene synthesis as a result of methyl group oxidation (Budavari et al., 1989). It is primarily used as an explosive, but has also had limited use in the vulcanization of rubber (Barnhart, 1981). 1,3,5-Trinitrobenzene and a similar compound, 1,3-dinitrobenzene (DNB), are used to produce plastics, herbicides, and paints and can enter domestic drinking water reservoirs via domestic effluent (Ryon et al., 1984; U.S. EPA, 1989). These compounds are not readily biodegradable and have a tendency to leach out into the ground water near production or disposal sites.

Fitzgerald et al. (1992) reported acute toxicity and irritancy data for TNB. The acute oral toxicity of TNB suspended in corn oil was reported as 298 and 275 mg/kg in male and female rats, and >900 and 702 mg/kg in male and female mice, respectively. No deaths occurred when neat TNB was in contact with rabbit skin for 24 h (2 g/kg limit test). 1,3,5-Trinitrobenzene was found to be a mild skin sensitizer in guinea pigs but did not cause acute irritation on rabbit skin. When applied as a powder, the treated eyes of all test rabbits were scored "severe" for redness, chemosis, and opacity through 96 h posttreatment. 1,3,5-Trinitrobenzene caused irreversible damage to ocular tissue and is considered to be corrosive.

Munition workers exposed to 2,4,6-trinitrotoluene (TNT) have developed skin irritation, liver damage, and anemia (Hathaway, 1977; Morton et al., 1976; Stewart et al., 1945). Animal studies have shown that oral treatment with structurally similar DNB or TNT causes anemia, increases methemoglobin concentration, and produces hypertrophy of the liver and spleen. Degeneration of the germinal epithelial lining of the seminiferous tubules also occurs, resulting in decreased spermatogenesis (Cody et al, 1981; Levine et al., 1983, 1984; Furedi et al., 1984a,b; Linder et al., 1986).

A range-finding reproduction study in which Sprague-Dawley rats received TNB-treated diet at concentrations of approximately 800, 400, and 67 mg TNB/kg diet resulted in testicular atrophy and sperm depletion in male rats receiving the two highest concentrations of diet (Kinkead et al., 1994). Head tilt and loss of

equilibrium occurred in the high-dose female rats during the postpartum period, and both sexes had brain lesions in the olivary region of the medulla at the conclusion of the 90-day study. Head tilt and loss of equilibrium (Linder et al., 1990), as well as brain and brain stem lesions (Philbert et al., 1987), have also been reported in rats following treatment with DNB.

SECTION 2

MATERIALS AND METHODS

Test Agent and Doses

The TNB/diet mixture was provided by the U.S. Army through a contract with the Environmental Protection Agency. Pertinent chemical and physical properties of the test compound are listed below:

1,3,5-Trinitrobenzene (TNB)

Synonyms:	Trinitrobenzene Benzite
CAS #:	99-35-4
Empirical Formula:	$C_6H_3N_3O_6$
Formula Weight:	213.11
Vapor Pressure	3.2×10^{-6} mmHg at 20 °C

The TNB was administered orally by mixing the test material into ground Purina Formulab #5002 (Ralston Purina, St. Louis, MO) certified rodent diet meal. Mean concentrations (and ranges) for each test concentration were 287 (278-305), 147 (138-158), and 30.7 (29.4-31.9) mg TNB/kg diet (five batches per treatment level). The TNB-diet preparation and analysis methods are summarized in Appendix A.

Group Assignments and Dose Levels

Group	Number of Animals Male	Female	Target Dose Level of TNB (mg/kg diet)	Target Dose of TNB (mg/kg body weight/day)[a]
Control	18	12	0.0	0.0
Low	18	12	30.0	1.8
Middle	18	12	150.0	9.0
High	18	12	300.0	18.0

[a]Assume 500 g rat consumes 30 g feed/day.

Test Animals and Clinical Measurements

Seventy-five male and 50 female Sprague-Dawley derived outbred albino rats [Crl:CD^R(sd)BR], known as Charles River CD rats, were purchased from Charles River Breeding Laboratories, Raleigh, NC. The rats were 9 weeks of age upon arrival and

11 weeks of age at initiation of the treatment period. All rats were identified by tail tattoo and were acclimatized for two weeks. During the acclimation period, quality control procedures were performed on selected rats as described in Kinkead et al. (1991). Rodent water and feed were available *ad libitum*. Animal room temperatures were maintained at 21 to 25 °C, and the light/dark cycle was set at 12-h intervals. Parental rats were single housed (except for the mating period) in clear plastic cages with hardwood-chip bedding (Bettachip^{R}, Northeastern Products Corp., Warrensburg, NY). During the mating period the animals were housed in clear plastic cages with stainless steel wire bottoms. There were four study groups with target doses of 0, 30, 150, and 300 mg TNB/kg diet. Rats were assigned to groups consisting of 12 females and 18 males by means of a computer-generated randomization. The randomization was stratified by body weight such that the mean body weights of all groups were homogeneous by statistical analysis at study initiation. Six male rats per group were maintained for two-months posttreatment on standard rodent diet (no TNB) to determine recovery of male reproductive effects.

Rats were observed twice daily for signs of toxic stress. Male rat body weights were measured weekly during the 90-day study, then biweekly during the posttreatment period. Female body weights were measured in the same manner until confirmation of mating. During gestation, females were weighed on Gestation Days 0, 4, 7, 14, and 20. Dams producing litters were weighed on Postpartum Days 0, 4, 7, 14, and 21, then weekly thereafter.

Food consumption was determined during the prebreeding period for both male and female rats. Food consumption of individual dams was measured for Gestation Days 0 to 7, 7 to 14, and 14 to 20, and for Postpartum Days 0 to 7 and 7 to 14. Male food consumption was calculated weekly through 90 days. Food consumption was not measured during the mating period when more than one rat was in a cage, during Postpartum Days 14 to 21 when pups were beginning to eat from the feed jars, or during the posttreatment period. Feed jars were cleaned on a weekly basis at which time all leftover food was discarded. Food consumption was measured every two/three days until the postpartum period when it was measured daily in the female rats. The live and dead pups were counted and sexed on Postpartum/Lactation Day 0. All pups were counted and sexed, and live pups were weighed 1, 4, 7, 14, and 21 days after birth. Standardization of litter sizes, 4 per sex when possible, occurred on Day 4. Pups were examined for external abnormalities.

General Study Design

Six male rats per group were dosed from 14 days prior to mating and throughout the mating period for a total of 28 days. A second group of six were treated for 90 days, and a third group, also treated for 90 days, were given control (untreated) diet for 2 months following the 90 days of treatment. All female rats were dosed from 14 days prior to mating, during mating and gestation, postpartum (21 days), and for 4 weeks postweaning for a total of 90 days. Pups were maintained on treated diet through 4-weeks postweaning.

One male and one female were cohabited, selected from within their respective dose groups, starting on Day 14. The pairs remained cohabited for up to 14 days, until either a copulation plug was present or sperm were present in the vaginal wash. The day a copulation plug was present or sperm were found in the vaginal wash was defined as Gestation Day 0.

At necropsy, blood samples were taken via the vena cava from fasted parental animals for hematology and clinical chemistry assays listed in Table 1. Erythrocytes were enumerated on a Coulter counter (Coulter Electronics, Hialeah, FL) and sera for clinical chemistry evaluations were assayed on an Ektachem 700XR (Eastman Kodak, Rochester, NY). Selected hematological parameters and absolute leukocyte differentials were determined according to established procedures. Sera were processed according to the procedures in the Ektachem Operations manual. Methemoglobin assays were performed using a Model IL282 cooximeter (Instrumentation Laboratory, Lexington, MA).

Opto-Varimex open-field activity evaluation tests (Columbus Instruments International Corporation, Columbus, OH) were performed on the parental rats. Tests were performed on male rats prior to dosing, postmating, and prior to sacrifice; on dams prior to dosing, during the postpartum period, and again prior to sacrifice. Four test chambers were used in the Opto-Varimex test, each having an observation area of approximately 17 x 17.5 inches, with sensors at 1-inch intervals (15 per side). Exposure sessions consisted of five consecutive 2-min intervals. Measurements included total distance traveled, time spent resting, time ambulatory, and time spent in "stereotypic movement" which included rearing and circling. The number of clockwise and counterclockwise rotations were tabulated. Following each session, the chambers were sprayed with a disinfectant/deodorant and wiped clean.

Evaluations at Necropsy

Brain, liver, kidneys, spleen, thymus, testes, and epididymides were weighed at necropsy. Sperm count and motility were also evaluated. Sperm were removed from the right cauda epididymis and analyzed microscopically using a videomicrography system (Cell Soft Automated Semen Analyzer, Cryo Resources, Ltd., Montgomery, NY) (Toth et al., 1992). Bouin's fixative was used to fix the testes and epididymides. The pituitary, spleen, liver, kidneys, bone marrow, and reproductive organs were removed from parental animals of both sexes and fixed in 10% buffered formalin solution. After routine processing, the tissues were embedded in paraffin and stained with hematoxylin and eosin for histopathologic examination. Pups were examined for gross lesions at necropsy.

Statistical Analysis

Maternal body weights, pup weights, organ weights, organ weight ratios, serum chemistry, hematology, and TNB dose calculations were analyzed for statistical significance using a one-factorial analysis of variance with Bonferroni multiple comparisons (Rosner, 1990). A one-factorial repeated measures analysis of variance with Bonferroni multiple comparisons was used for food consumption and paternal body weights (Barcikowski, 1983). Mating indices and histopathologic results were analyzed by a chi-square test of proportions applied to the incidence data (Rosner, 1990). Tissue lesion severity data were analyzed using the Kruskal-Wallis analysis of variance (Rosner, 1990).

Sperm analysis and Opto-Varimex data were analyzed using one-way analysis of variance, employing Dunnett's technique (Sokal and Rohlf, 1981) for multiple comparisons between controls and treatments when significant differences ($p<0.05$) occurred. Parametric analysis techniques were performed. However, when transformation techniques failed to present a normal distribution, a Kruskal-Wallis rank-based analysis of variance (Sokal and Rohlf, 1981) was used.

SECTION 3

RESULTS

Food Consumption and Calculated Dose

Food consumption (Tables 2 and 3) decreased significantly in the high- and mid-dose groups of both sexes when treatment began. Food consumption returned to pretreatment levels after four days. Food consumption of the female rats increased during the postpartum (lactation) period compared to the premating and gestation periods. Male rats consumed approximately 30 g diet/day which resulted in a dose of approximately 19, 9, and 2 mg TNB/kg/day in the high-, mid-, and low-dose groups, respectively (Figure 1). Female rats consumed an average of 30 g diet/day which converts to approximately 29, 14, and 3 mg TNB/kg/day for the high-, mid-, and low-dose groups, respectively (Figure 2).

General Toxicity

No mortality occurred in the parental animals during the study. Mean body weights of the high-dose male rats were significantly less than the mean body weights of the control group beginning on Day 21 and continuing through the 90-day treatment period (Figure 3 and Appendix B). All male rat groups maintained posttreatment showed increased weight gain when returned to untreated diet for the final two months; however, the mean body weight of the high-dose group, although not significantly different, remained lower than the control group. Mean body weights of the high-dose female rats were slightly less than the control group throughout the study; however, the difference was statistically significant only at Lactation Day 0 (Figure 4 and Appendix C).

No clinical signs of motor skill loss were noted during the study. This was confirmed by the Opto-Varimex tests in which no differences in locomotor skills were noted in either treated or control animals.

Six male rats per group were necropsied following the mating period (28 days of treatment), after 90 days of treatment, and following a two-month recovery period. No treatment-related differences were noted in absolute or relative organ weights in male rats at any of the evaluation periods (Tables 4 through 6). An increase in absolute and relative spleen weights was noted in the high-dose female rats necropsied following 90 days of treatment. Relative liver weights were increased in the high-dose group and relative kidney weights were increased in both the high- and mid-dose female groups (Table 7).

High-dose male rats, sacrificed following mating (28 days) and after 90 days of treatment, showed adverse effects for most measurements of sperm function/activity (Table 8). The number and concentration of motile cells were greatly reduced in the high-dose group. The percent of cells traveling in a circular pattern was reduced in both the high-dose and the mid-dose groups after 28 and 90 days of treatment. Sperm function/activity evaluated in the TNB-treated rats maintained on control diet for two months was similar to the sperm function of control rats (Table 8).

Methemoglobin concentration was significantly ($p<0.01$) increased in the high- and mid-dose rats of both sexes following either 28 or 90 days of treatment (Tables 9 through 11). A significant decrease in percent neutrophils and significant increase in lymphocytes was noted in the high- and mid-dose female rats after 90 days. The high-dose female rats also showed a significant decrease in hemoglobin values. Male rats which were maintained posttreatment on control diet had methemoglobin values similar to those of the control group (Table 12).

After 28 days of treatment, calcium levels were increased in the high-dose male rat group and alkaline phosphatase values were low in the low-dose male rat group (Table 13). There were no abnormalities noted in the selected clinical chemistry parameters measured in male rats following 90 days of treatment or after the 2-month recovery period (Tables 14 and 15). Cholesterol values were significantly increased in the high-dose female rat group and LDH values were significantly decreased in the mid-dose female rat group at the conclusion of the study (Table 16).

At necropsy all animals used in the study were in good general condition. Dilation of the uterus by clear fluid was noticed in two rats from both the high- and mid-dose groups, and in one rat from the low-dose group. This finding was not observed in any of the control female rats. Discoloration of the kidneys was observed exclusively in males involving two to three animals per group, including controls. Other nontreatment-related gross observations included testicular atrophy, splenomegaly, and discoloration of the thymus and liver (each observed only once). Gross examination of the pups revealed foci of tan discoloration in the liver of one animal from the control group.

Histopathology

Observed histopathologic lesions of statistical, clinical, or pathologic significance were limited to the spleen, kidney, and testes (Tables 17 through 20). After 28 days of treatment, severity and incidence of splenic hemosiderosis

was significantly increased in the mid- ($p<0.05$) and high-dose ($p<0.01$) male rats. At 90 days, the severity of splenic hemosiderosis was increased ($p<0.01$) in the high-dose males when compared to controls. The severity of splenic hemosiderosis was also significantly ($p<0.01$) increased in the mid- and high-dose female rats examined after 90 days.

There was a significant decrease in the incidence and severity of renal interstitial lymphoid infiltrates in the mid-dose male rats examined after 28 days of treatment. At 90 days, there was increased incidence and severity of hyaline droplets in all TNB-treated male rats. There was also an increased ($p<0.05$) severity in renal lymphoid filtrates in the high-dose male rats. Severity and incidence of hyaline droplet change was also significantly increased ($p<0.01$) in the high-dose female rats after 90 days.

There was a significant increase in the incidence and severity of testicular seminiferous tubular degeneration after 90 days of treatment, and after two-months recovery, that was not noted after 28 days of treatment. There was also a corresponding increased ($p<0.05$) incidence of sperm abnormality seen in the epididymides of the high-dose rats following 90 days of treatment. However, there was recovery after two months, as both incidence and severity of the lesions were reduced.

Reproductive Indices

The treatment showed no adverse effects on mating as 100% of the animals mated (Table 21). The fertility index was 92% in the high-dose group and 100% in the other treated and control groups. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, the mean body weights of the TNB-treated pups, both male and female, were significantly lower than the control group pups (Figure 5 and Appendix D) except at 14 days, when the mid- and low-dose pup weights were equivalent to controls. Because no differences in mean body weights were noted between sexes of pups during the 21-day period, the sexes were combined for statistical analyses.

SECTION 4

DISCUSSION

Administering TNB in the diet of Sprague-Dawley rats at calculated dose levels of approximately 19, 9, and 2 or 29, 14, and 3 mg TNB/kg body weight/day of males and females, respectively, produced no adverse effects on reproductive performance or litter parameters. Treatment-related decreases in mean body weights of pups (as great as 15% in the high-dose group) was observed during the 21-day postpartum period.

Anemia and increased methemoglobin production are common characteristics of nitrate poisoning. Although anemia was not noted in this study, the increased incidence and/or severity of splenic hemosiderosis suggests an increased removal (phagocytosis) of erythrocytes. The change in leukocyte parameters in the mid- and high-dose female rats as well as alteration in cholesterol values in the high-dose females is not believed to be biologically significant.

Brain lesions or motor skills loss were not noted following treatment at these dose levels as was noted following treatment with DNB or higher doses of TNB (Linder et al., 1990; Kinkead et al., 1994). However, sperm depletion and degeneration of the seminiferous tubules at the high-dose level during the treatment period was clearly a treatment-related effect, typical of nitrate toxicity (Linder et al., 1986, 1990; Cody et al., 1981). Sperm loss and degeneration had no effect on mating as the fertility index of the TNB-treated rats was >90%. Full recovery from testicular lesions did not occur within 60 days after removal from TNB-treated diet; however, sperm concentration did return to control levels.

In summary, TNB has been shown to disrupt spermatogenic activity at doses as low as 9 mg TNB/kg/day, but the testicular effects are not nearly as potent as with DNB. Although the sperm concentration in TNB-treated rats was reduced, fertility of the male rats appeared to be unaffected.

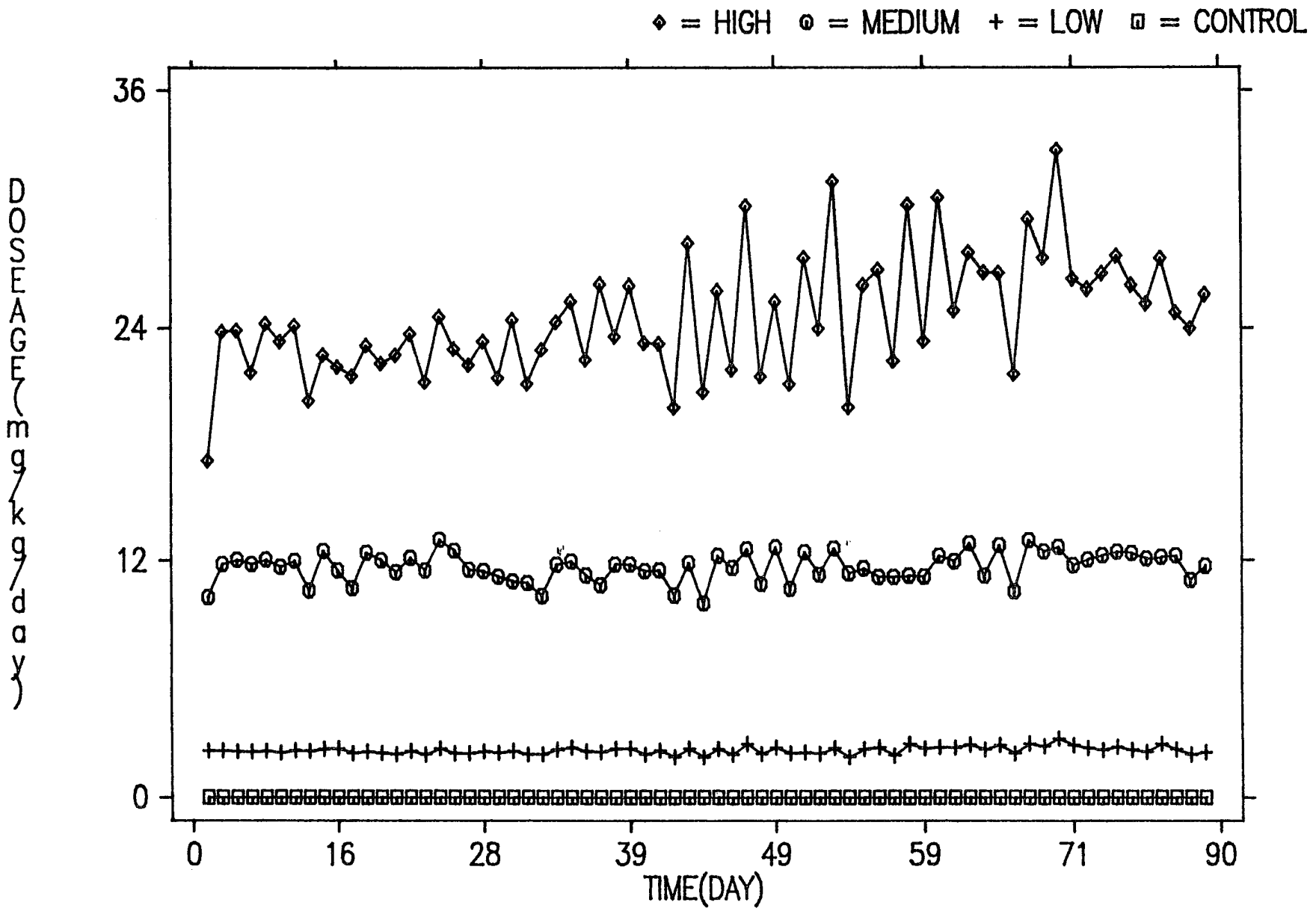

Figure 1. Mean Dose of Male Rats During 90-Day Treatment with TNB.

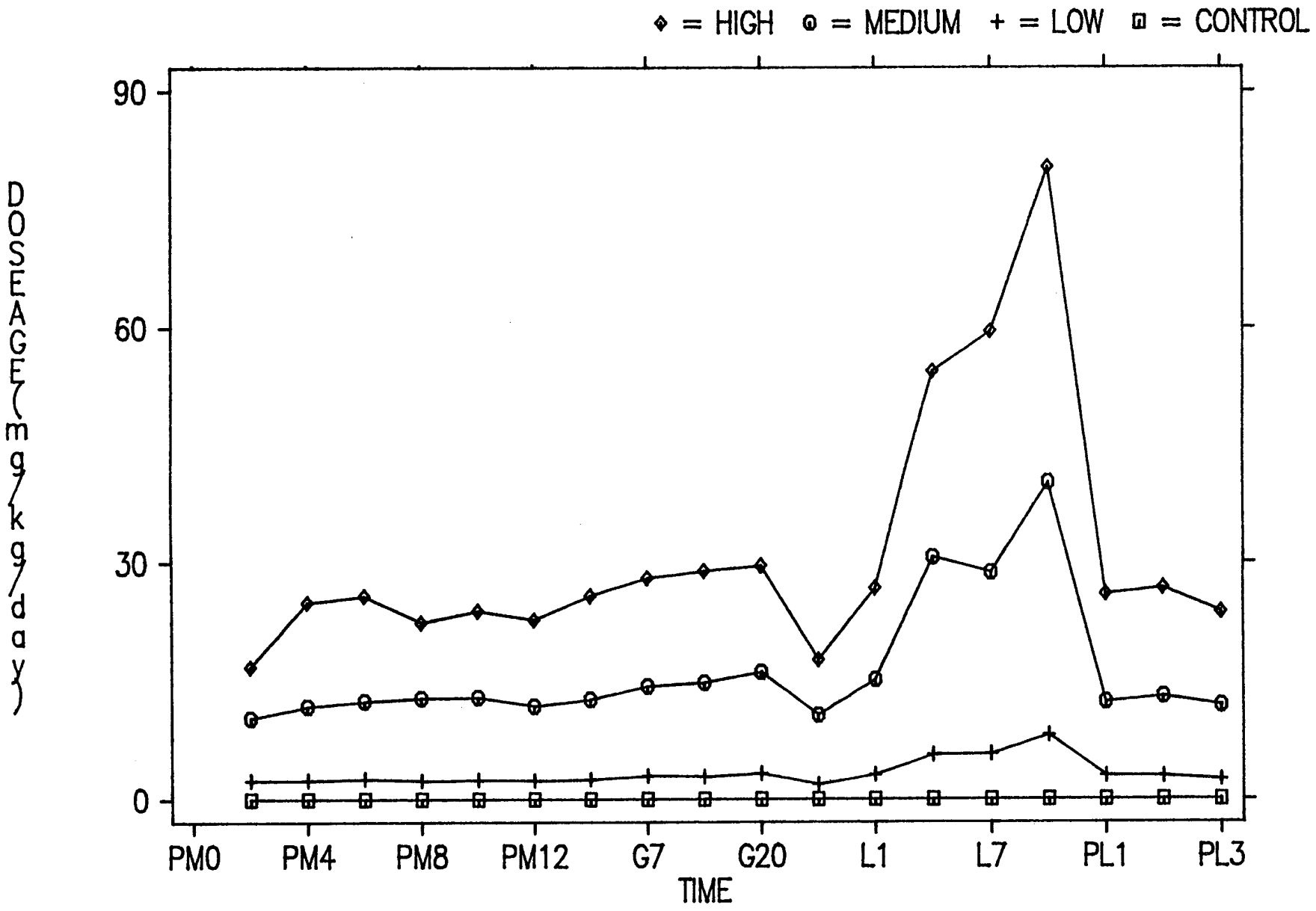

Figure 2. Mean Dose of Female Rats During 90-Day Treatment with TNB.

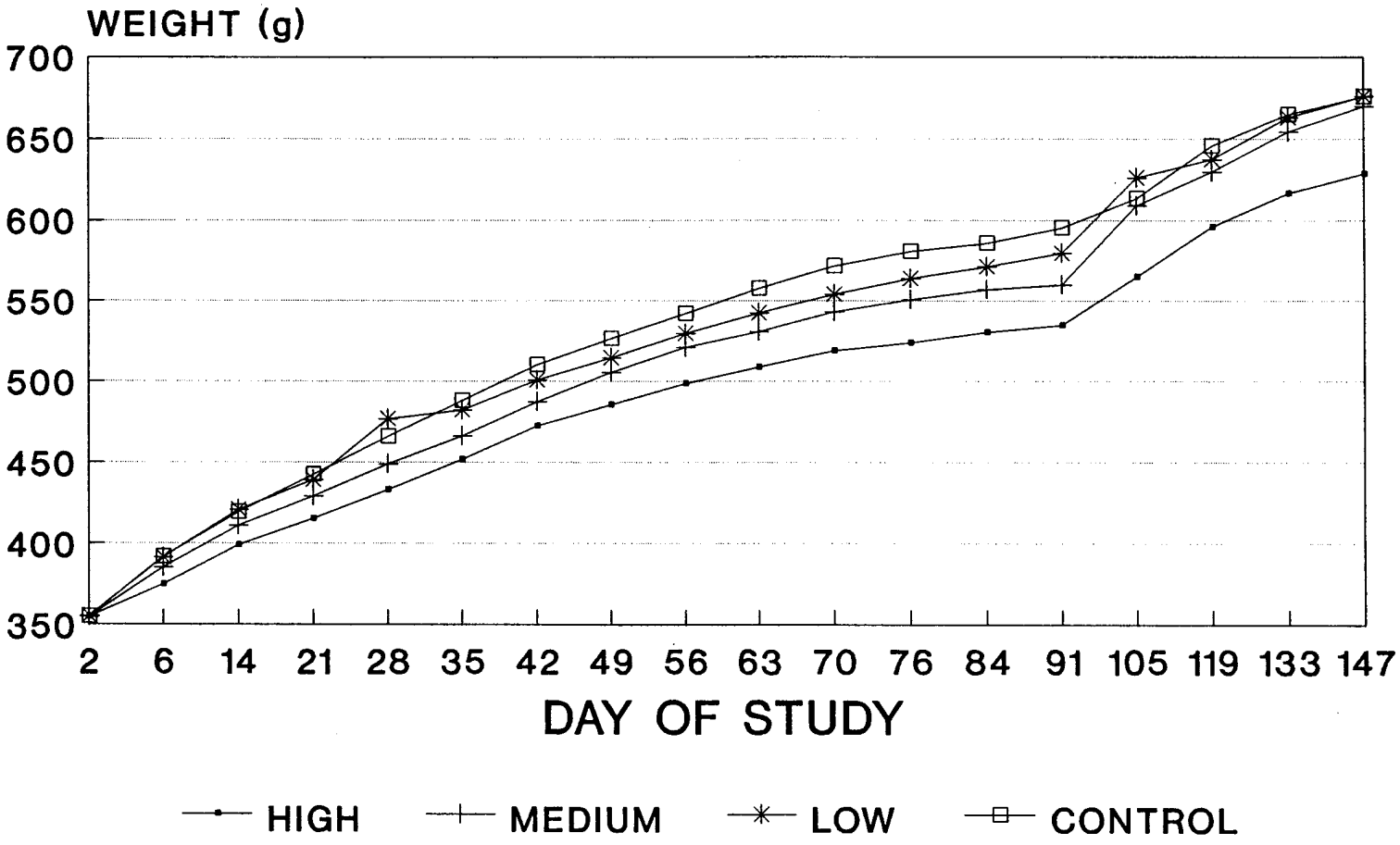

Figure 3. Mean Body Weights of Male Rats.

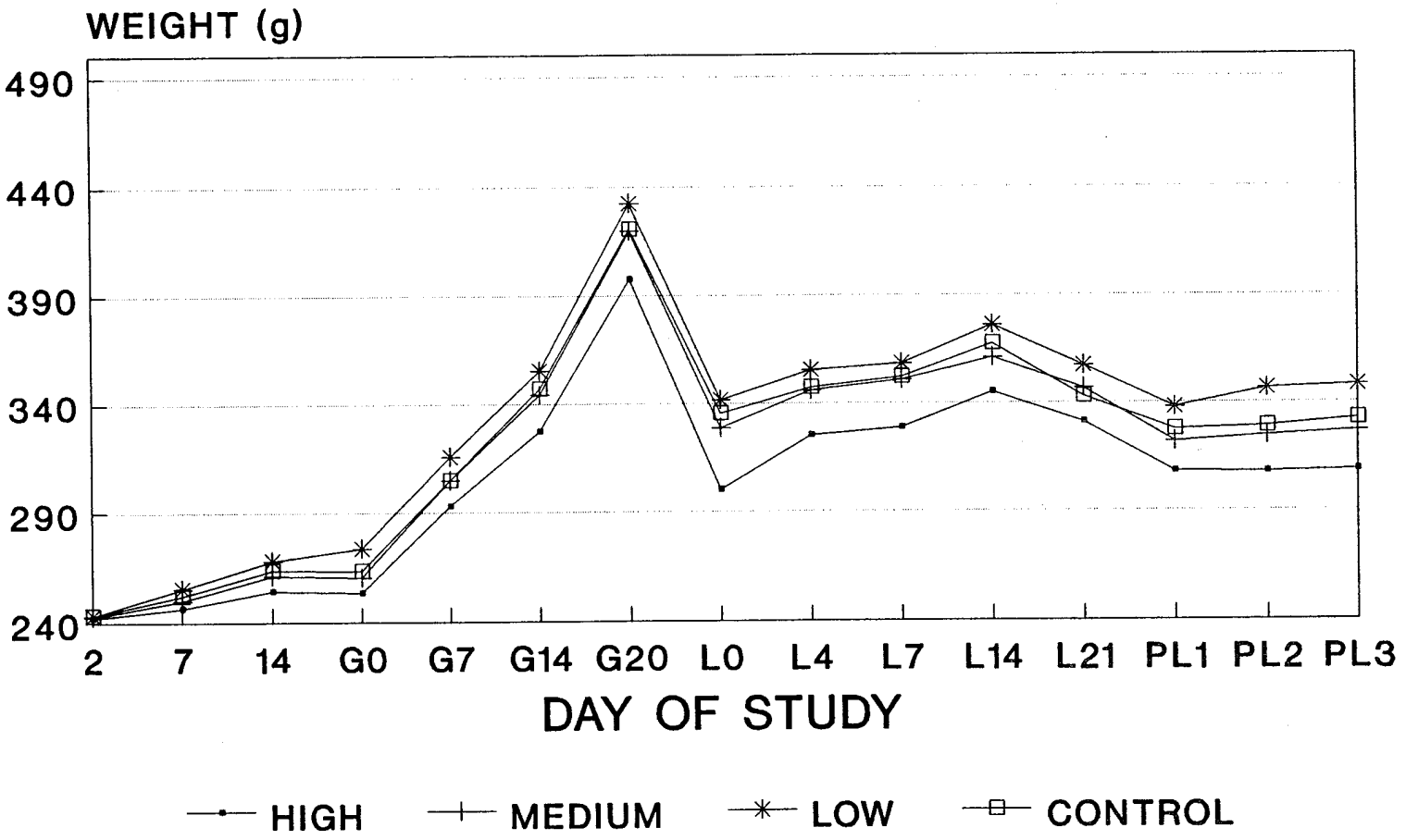

Figure 4. Mean Body Weight of Female Rats.

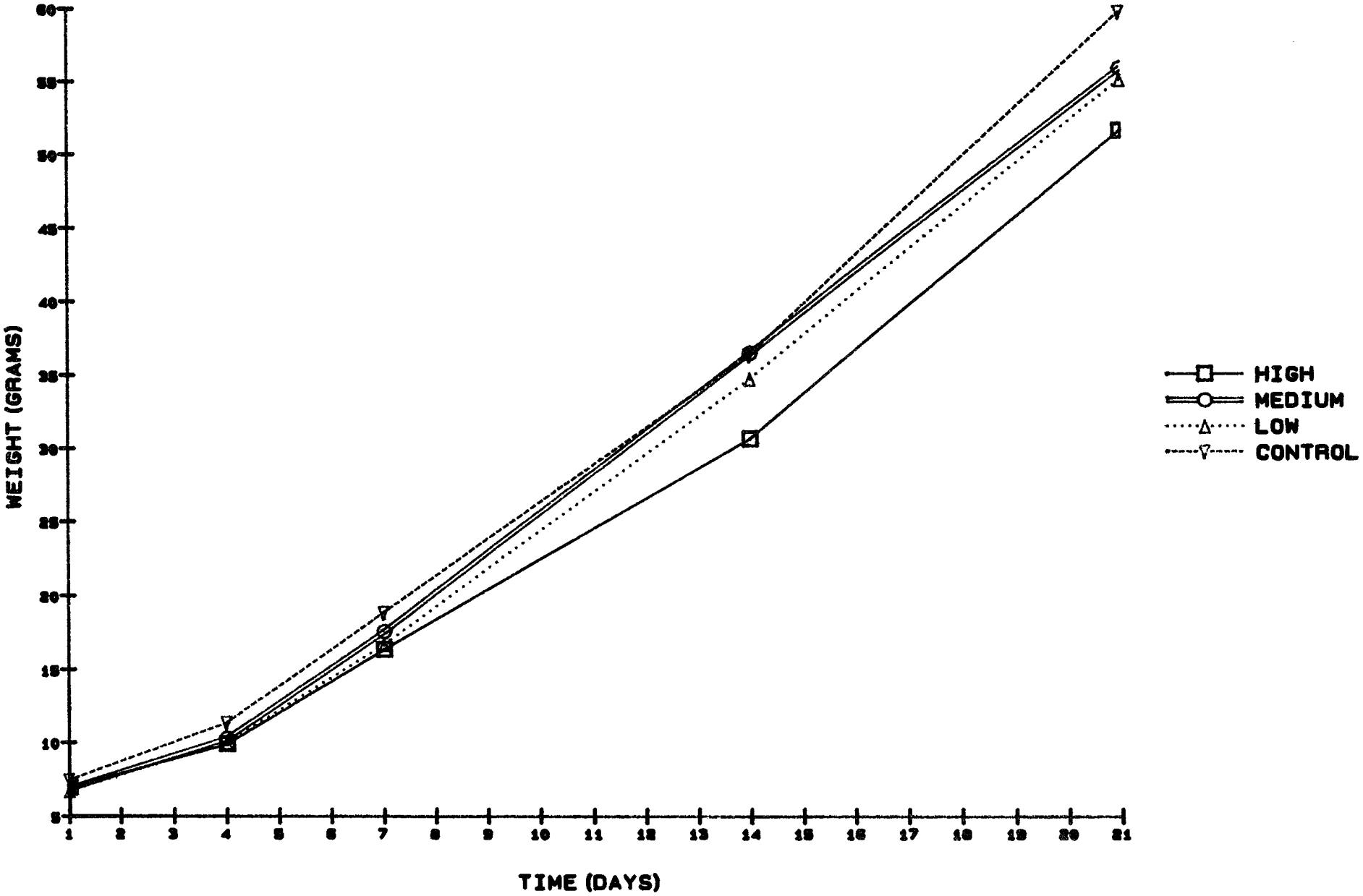

Figure 5. Pup Mean Body Weights.

Table 1. Serum Chemistry and Whole Blood Assessments from Control and TNB-Treated Sprague-Dawley Rats

Albumin	Magnesium
Alkaline phosphatase	Triglycerides
Alanine aminotransaminase	Cholesterol
Aspartate aminotransaminase	Hematocrit
Bilirubin	Hemoglobin
Blood Urea Nitrogen	Red blood cell count
Creatinine	Total and differential leukocyte count
Chloride	Platelet count
Calcium	
Glucose	
Potassium	
Phosphorus	
Sodium	
Total protein	

Table 2. Mean Food Consumption of Male Rats Treated with TNB for 90 Days

DAY	CONTROL	LOW	MEDIUM	HIGH
-5	29.6 ± 0.9	28.5 ± 0.8	29.5 ± 0.7	33.0 ± 2.8
-3	28.5 ± 0.4	28.9 ± 0.9	30.2 ± 1.3	29.8 ± 1.3
-1	29.4 ± 0.7	29.0 ± 0.7	30.0 ± 0.8	29.8 ± 0.8
2	28.6 ± 0.6	28.4 ± 0.7	24.2 ± 1.4[a]	19.8 ± 1.0[b]
4	29.3 ± 0.7	28.6 ± 0.7	28.2 ± 0.7	27.6 ± 1.0
6	28.8 ± 0.5	28.0 ± 0.6	28.7 ± 0.9	27.6 ± 0.8
8	28.6 ± 0.8	27.9 ± 0.7	28.2 ± 0.9	25.1 ± 0.6[b]
10	29.0 ± 0.8	28.4 ± 0.5	28.8 ± 0.7	28.0 ± 0.7
12	27.9 ± 0.7	27.4 ± 0.9	27.8 ± 0.9	27.0 ± 0.8
14	29.5 ± 0.7	28.5 ± 0.6	28.7 ± 1.0	27.9 ± 1.0
15	27.3 ± 0.9	28.4 ± 2.0	24.8 ± 1.6	24.0 ± 1.3
16	31.0 ± 1.3	29.1 ± 1.5	29.8 ± 1.5	26.4 ± 1.3
17	27.3 ± 0.8	29.4 ± 1.2	27.7 ± 1.4	25.4 ± 1.7
18	26.4 ± 1.2	26.3 ± 1.0	25.4 ± 0.9	25.0 ± 0.9
19	29.9 ± 1.1	27.9 ± 1.0	29.6 ± 1.1	26.6 ± 1.3
20	28.8 ± 1.5	27.1 ± 0.8	28.7 ± 1.1	25.6 ± 1.1
22	26.6 ± 1.1	26.3 ± 0.8	27.3 ± 0.9	26.1 ± 0.9
23	27.9 ± 0.7	28.1 ± 1.0	29.0 ± 1.1	27.4 ± 1.0
24	25.0 ± 1.0	26.2 ± 0.9	27.5 ± 1.9	24.5 ± 1.1
25	28.8 ± 0.8	29.9 ± 1.1	31.1 ± 2.0	28.4 ± 0.8
26	27.7 ± 1.0	26.7 ± 0.9	29.9 ± 3.0	26.4 ± 1.3
28	26.8 ± 0.6	26.9 ± 1.0	27.5 ± 0.9	25.5 ± 1.0
29	27.3 ± 0.9	28.3 ± 1.0	27.3 ± 1.5	26.8 ± 1.1
30	27.0 ± 0.6	27.5 ± 1.1	26.6 ± 1.2	24.7 ± 1.1
31	29.0 ± 1.3	28.5 ± 0.9	26.0 ± 0.6	28.1 ± 1.1
32	26.3 ± 1.0	26.4 ± 1.2	25.9 ± 0.8	24.3 ± 1.4
33	28.3 ± 1.9	26.5 ± 1.5	24.3 ± 1.0	26.4 ± 0.8
35	29.2 ± 1.0	29.5 ± 1.1	28.1 ± 1.1	28.0 ± 1.6
36	29.4 ± 1.0	30.6 ± 1.6	28.4 ± 0.9	29.2 ± 1.3
37	27.4 ± 1.5	28.2 ± 1.7	26.7 ± 0.6	25.8 ± 1.0
38	30.1 ± 1.7	28.0 ± 1.1	25.6 ± 1.1	30.3 ± 2.0
39	27.2 ± 0.8	29.8 ± 1.0	28.1 ± 0.8	27.1 ± 1.1
40	30.0 ± 1.1	30.1 ± 1.3	28.1 ± 0.7	30.1 ± 1.1
41	28.7 ± 0.9	26.3 ± 1.9	27.2 ± 1.6	26.7 ± 1.9
42	30.2 ± 2.7	28.9 ± 1.5	27.5 ± 2.0	26.8 ± 1.3
43	26.6 ± 0.9	24.8 ± 1.1	24.3 ± 1.0	22.9 ± 1.2
44	30.9 ± 0.8	30.1 ± 1.6	28.3 ± 1.0	32.5 ± 2.2
45	26.2 ± 1.7	24.6 ± 1.0	23.5 ± 1.0	23.8 ± 2.0
46	28.9 ± 0.9	29.8 ± 1.3	29.1 ± 1.3	29.8 ± 1.3
47	27.1 ± 0.9	26.6 ± 1.5	27.6 ± 1.2	25.2 ± 1.5
48	33.1 ± 1.6	33.1 ± 2.6	29.9 ± 1.3	34.7 ± 1.5
49	29.0 ± 1.8	26.6 ± 1.3	25.7 ± 1.0	24.7 ± 1.3
50	30.9 ± 1.0	30.8 ± 2.2	30.2 ± 2.5	29.1 ± 1.7

Table 2 cont'd

DAY	CONTROL	LOW	MEDIUM	HIGH
51	27.4 ± 1.1	27.3 ± 1.5	25.2 ± 1.2	24.4 ± 1.6
52	31.8 ± 1.6	27.9 ± 1.5	29.6 ± 1.8	31.8 ± 2.6
53	25.8 ± 1.0	26.8 ± 1.0	26.8 ± 0.8	27.6 ± 1.3
54	32.7 ± 1.9	30.3 ± 1.8	30.0 ± 1.6	36.0 ± 3.3
55	25.2 ± 0.8	24.7 ± 1.3	27.0 ± 1.4	22.8 ± 1.4
56	33.4 ± 2.1	29.8 ± 1.8	27.6 ± 1.2	30.1 ± 1.4
57	31.8 ± 1.5	30.7 ± 1.2	26.5 ± 1.0	31.0 ± 1.9
58	24.5 ± 1.2	25.8 ± 0.8[a]	26.6 ± 0.8	25.7 ± 0.9[a]
59	31.9 ± 1.6	33.0 ± 2.7	26.8 ± 0.9	34.7 ± 2.5
60	31.5 ± 1.5	30.1 ± 1.8	26.6 ± 1.2	26.9 ± 1.4
61	34.4 ± 2.5	30.8 ± 1.6	29.0 ± 1.5	35.2 ± 1.9
62	30.9 ± 1.2	30.5 ± 1.8	28.5 ± 1.2	28.7 ± 1.4
63	35.3 ± 2.6	32.4 ± 2.7	30.5 ± 1.8	31.9 ± 2.5
64	34.8 ± 2.1	29.6 ± 1.7	26.7 ± 1.2	30.7 ± 2.9
65	33.3 ± 1.2	32.3 ± 2.1	30.3 ± 1.5	30.8 ± 1.2
66	27.8 ± 2.0	27.1 ± 1.5	24.8 ± 1.4	24.9 ± 1.6
68	33.6 ± 1.3	33.1 ± 1.1	31.0 ± 1.5	33.9 ± 1.6
70	33.4 ± 1.0	31.3 ± 1.7	29.5 ± 1.2	31.6 ± 1.5
71	32.4 ± 2.6	35.9 ± 3.3	30.1 ± 1.8	38.0 ± 2.4
72	32.2 ± 2.3	32.0 ± 2.7	28.0 ± 0.9	30.4 ± 2.4
74	32.0 ± 1.6	30.3 ± 1.5	28.6 ± 1.4	29.8 ± 1.7
76	30.8 ± 1.5	29.0 ± 1.2	29.1 ± 1.1	30.8 ± 1.4
78	33.4 ± 1.4	30.9 ± 1.8	29.5 ± 1.8	31.8 ± 2.0
80	30.5 ± 1.3	29.7 ± 1.8	29.3 ± 1.4	30.1 ± 1.3
82	29.9 ± 1.1	28.1 ± 1.3	28.6 ± 1.4	29.1 ± 1.7
84	32.3 ± 1.4	32.7 ± 2.2	28.8 ± 2.0	31.6 ± 1.7
86	32.0 ± 1.5	29.3 ± 1.2	29.0 ± 1.0	28.5 ± 1.5
88	28.2 ± 1.5	26.3 ± 1.2	26.1 ± 1.0	27.6 ± 1.6
90	30.3 ± 1.9	27.8 ± 2.3	27.8 ± 2.5	29.6 ± 2.4

[a]Significantly different from control at $p<0.05$.
[b]Significantly different from control at $p<0.01$.

Table 3. Mean Food Consumption of Female Rats Treated with TNB for 90 Days

DAY	CONTROL	LOW	MEDIUM	HIGH
PD5	17.4 ± 1.1	18.3 ± 0.7	19.5 ± 0.7	18.4 ± 0.6
PD3	19.4 ± 1.1	20.3 ± 0.9	20.7 ± 0.9	20.7 ± 0.9
PD1	21.1 ± 0.8	20.5 ± 1.3	19.9 ± 1.0	19.0 ± 0.6
PM2	19.7 ± 0.8	19.7 ± 1.0	16.8 ± 0.7	13.5 ± 1.3[a]
PM4	20.9 ± 1.2	20.0 ± 0.5	19.2 ± 0.9	20.3 ± 1.3
PM6	20.6 ± 0.3	21.4 ± 0.7	20.2 ± 1.0	20.9 ± 1.1
PM8	19.3 ± 0.8	19.3 ± 0.6	20.9 ± 1.6	18.1 ± 1.2
PM10	20.2 ± 1.2	20.6 ± 0.9	21.0 ± 1.1	19.4 ± 0.9
PM12	19.2 ± 0.9	19.6 ± 0.9	19.2 ± 1.0	18.4 ± 0.8
PM14	19.0 ± 0.9	21.0 ± 1.0	20.7 ± 1.6	20.9 ± 1.1
G7	24.9 ± 1.1	24.7 ± 1.2	23.2 ± 1.4	22.9 ± 1.2
G14	25.0 ± 1.4	23.4 ± 1.2	23.9 ± 1.0	23.5 ± 0.9
G20	27.0 ± 0.9	17.0 ± 1.0	26.1 ± 0.8	24.1 ± 1.4
LO	19.9 ± 1.6	15.3 ± 1.4	17.2 ± 1.4	14.4 ± 1.6
L1	23.4 ± 2.2	25.9 ± 1.7	24.6 ± 2.4	21.9 ± 2.7
L4	46.8 ± 2.4	46.2 ± 2.5	49.6 ± 2.8	44.1 ± 1.5
L7	50.6 ± 2.6	46.8 ± 2.1	46.3 ± 1.6	48.3 ± 1.4
L14	68.6 ± 1.8	66.4 ± 2.3	64.3 ± 2.7	65.3 ± 2.1
PW1	23.6 ± 1.2	24.3 ± 2.0	20.0 ± 0.9	21.2 ± 1.8
PW2	23.0 ± 2.0	24.0 ± 1.1	21.2 ± 1.0	21.7 ± 1.4
PW3	22.1 ± 1.6	20.3 ± 1.0	19.3 ± 0.9	19.2 ± 1.2

[a]Significantly different than control at $p<0.01$.

PD = Pretreatment PM = Premating
G = Gestation L = Lactation
PW = Postweaning

Table 4. Absolute and Relative Organ Weights[a] of Male Rats Treated with TNB for 28 Days

	CONTROL	LOW	MEDIUM	HIGH
Testes	3.60 ± 0.12	3.65 ± 0.03	3.50 ± 0.08	3.36 ± 0.14
Ratio[b]	0.82 ± 0.03	0.81 ± 0.03	0.82 ± 0.02	0.81 ± 0.03
Epididymis	1.38 ± 0.06	1.35 ± 0.03	1.28 ± 0.04	1.24 ± 0.03
Ratio	0.31 ± 0.01	0.30 ± 0.01	0.30 ±<0.01	0.30 ± 0.01
Brain	2.13 ± 0.04	2.12 ± 0.03	2.02 ± 0.03	2.11 ± 0.05
Ratio	0.49 ± 0.01	0.47 ± 0.02	0.47 ± 0.02	0.51 ± 0.01
Liver	13.96 ± 0.90	14.96 ± 0.65	14.57 ± 0.71	13.42 ± 0.64
Ratio	3.16 ± 0.12	3.32 ± 0.13	3.39 ± 0.09	3.24 ± 0.09
Kidneys	3.40 ± 0.12	3.68 ± 0.13	3.68 ± 0.18	3.52 ± 0.12
Ratio	0.77 ± 0.01	0.82 ± 0.03	0.86 ± 0.03	0.85 ± 0.02
Spleen	0.92 ± 0.12	0.84 ± 0.04	0.85 ± 0.07	0.84 ± 0.02
Ratio	0.21 ± 0.02	0.19 ± 0.01	0.20 ± 0.01	0.20 ±<0.01
Thymus	0.46 ± 0.04	0.47 ± 0.03	0.51 ± 0.05	0.46 ± 0.03
Ratio	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Body Wt	440.4 ± 16.3	451.3 ± 12.5	428.5 ± 12.5	413.6 ± 10.7

[a]Mean ± SEM, N=6.
[b]Organ weight/body weight x 100.

Table 5. Absolute and Relative Organ Weights[a] of Male Rats Treated with TNB for 90 Days

	CONTROL	LOW	MEDIUM	HIGH
Testes	3.73 ± 0.08	3.67 ± 0.08	3.65 ± 0.16	3.25 ± 0.24
Ratio[b]	0.66 ± 0.02	0.69 ± 0.02	0.68 ± 0.03	0.63 ± 0.06
Epididymis	1.54 ± 0.05	1.49 ± 0.06	1.51 ± 0.05	1.32 ± 0.08
Ratio	0.27 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.26 ± 0.02
Brain	2.17 ± 0.04	2.13 ± 0.02	2.16 ± 0.07	2.13 ± 0.02
Ratio	0.38 ± 0.01	0.40 ± 0.01	0.40 ± 0.02	0.41 ± 0.02
Liver	16.81 ± 0.54	15.71 ± 0.77	15.98 ± 0.98	16.29 ± 0.86
Ratio	2.95 ± 0.09	2.92 ± 0.07	2.95 ± 0.10	3.13 ± 0.07
Kidneys	3.88 ± 0.15	4.04 ± 0.14	4.05 ± 0.28	4.14 ± 0.19
Ratio	0.68 ± 0.03	0.75 ± 0.02	0.75 ± 0.03	0.80 ± 0.03
Spleen	0.83 ± 0.06	0.83 ± 0.04	0.99 ± 0.08	0.96 ± 0.06
Ratio	0.15 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Thymus	0.47 ± 0.05	0.45 ± 0.07	0.45 ± 0.05	0.42 ± 0.04
Ratio	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.08 ±<0.01
Body Wt	569.8 ± 9.26	535.9 ± 13.6	539.8 ± 17.4	519.6 ± 17.4

[a]Mean ± SEM, N=6.
[b]Organ weight/body weight x 100.

Table 6. Absolute and Relative Organ Weights[a] of Male Rats Treated with TNB, 2-Months Posttreatment

ORGAN	CONTROL	LOW	MEDIUM	HIGH
Liver	20.62 ± 3.75	21.03 ± 2.54	21.17 ± 2.22	18.22 ± 2.50
Ratio[b]	3.08 ± 0.30	3.13 ± 0.14	3.19 ± 0.13	2.95 ± 0.40
Kidneys	4.36 ± 0.21	4.85 ± 0.52	4.72 ± 0.47	4.28 ± 0.50
Ratio	0.65 ± 0.02	0.73 ± 0.11	0.71 ± 0.06	0.69 ± 0.09
Testes	3.78 ± 0.20	3.76 ± 0.27	3.96 ± 0.41	3.62 ± 0.24
Ratio	0.57 ± 0.05	0.57 ± 0.07	0.60 ± 0.03	0.64 ± 0.04
Brain	2.18 ± 0.08	2.20 ± 0.10	2.30 ± 0.14	2.20 ± 0.06
Ratio	0.33 ± 0.03	0.33 ± 0.03	0.35 ± 0.03	0.36 ± 0.03
Spleen	0.99 ± 0.18	0.93 ± 0.12	1.06 ± 0.17	0.91 ± 0.14
Ratio	0.15 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.02
Thymus	0.52 ± 0.14	0.50 ± 0.15	0.53 ± 0.10	0.43 ± 0.08
Ratio	0.08 ± 0.02	0.07 ± 0.02	0.08 ± 0.01	0.07 ± 0.01
Epididymis	1.71 ± 0.16	1.68 ± 0.07	1.66 ± 0.22	1.56 ± 0.13
Ratio	0.26 ± 0.02	0.25 ± 0.03	0.25 ± 0.03	0.25 ± 0.00

[a]Mean ± SD, N=6.
[b]Organ weight/body weight x 100.

Table 7. Absolute and Relative Organ Weights[a] of Female Rats Treated with TNB for 90 Days

ORGAN	CONTROL	LOW	MEDIUM	HIGH
Liver	9.87 ± 0.32	10.21 ± 0.48	10.05 ± 0.24	9.91 ± 0.35
Ratio[b]	3.06 ± 0.06	3.03 ± 0.08	3.22 ± 0.07	3.34 ± 0.07[c]
Spleen	0.60 ± 0.01	0.63 ± 0.03	0.66 ± 0.02	0.69 ± 0.02[c]
Ratio	0.19 ±<0.01	0.19 ± 0.01	0.21 ± 0.01	0.23 ± 0.01[d]
Kidneys	2.16 ± 0.06	2.26 ± 0.08	2.27 ± 0.06	2.17 ± 0.06
Ratio	0.67 ± 0.01	0.67 ± 0.01	0.73 ± 0.01[c]	0.73 ± 0.01[c]
Brain	2.01 ± 0.02	1.98 ± 0.09	1.97 ± 0.03	1.95 ± 0.03
Ratio	0.63 ± 0.02	0.59 ± 0.02	0.63 ± 0.01	0.66 ± 0.01
Thymus	0.34 ± 0.02	0.42 ± 0.03	0.37 ± 0.03	0.37 ± 0.02
Ratio	0.11 ±<0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Body Wt	321.8 ± 7.3	336.4 ± 10.2	313.4 ± 8.0	296.4 ± 6.1

[a]Mean ± SEM, N=12.
[b]Organ weight/body weight x 100.
[c]Statistically different from control at $p<0.01$.
[d]Statistically different from control at $p<0.05$.

Table 8. Sperm Evaluations from Rats Administered TNB in Diet

Parameter	Control (N=6)	Low (N=6)	Mid (N=6)	High (N=6)
28 Days of Treatment				
Mean (± SEM) Number Motile Cells Analyzed	406.5 ± 26.1	383.2 ± 36.9	329.7 ± 28.8	256.5 ± 33.1[a]
Concentration Motile (million/mL)	1.74	1.12	1.05	0.83[a]
Mean (± SEM) Number of Cells Traveling in a Circular Pattern	87.5 ± 8.0	90.8 ± 4.2	73.0 ± 4.2[b]	52.3 ± 6.9[a]
Percent Cells Traveling in a Circular Pattern	16.0	17.8	15.9	11.3[b]
Percent in Circular Pattern Compared to Total Motile Cells	21.5	24.4	22.6	20.4
90 Days of Treatment				
Mean (± SEM) Number Motile Cells Analyzed	497.7 ± 47.7	504.8 ± 59.3	361.5 ± 67.8	229.0 ± 75.8[a]
Concentration Motile (million/mL)	1.69	1.67	1.37	0.78[a]
Mean Number of Cells Traveling in a Circular Pattern	84.3 ± 6.0	87.8 ± 10.9	60.8 ± 11.3[b]	55.6 ± 13.9[a]
Percent Cells Traveling in a Circular Pattern	11.7	11.7	10.0[b]	10.0[b]
Percent in Circular Pattern Compared to Total Motile Cells	17.3	17.4	16.9	24.5[b]
2-Months Posttreatment				
Mean (± SEM) Number Motile Cells Analyzed	380.8 ± 34.4	321.2 ± 55.2	375.3 ± 13.5	343.2 ± 29.6
Concentration Motile (million/mL)	1.19	1.03	1.20	1.03
Mean Number of Cells Traveling in a Circular Pattern	87.3 ± 5.9	68.3 ± 13.2	86.0 ± 3.6	79.5 ± 6.8
Percent Cells Traveling in a Circular Pattern	19.1	13.3	18.3	17.0
Percent in Circular Pattern Compared to Total Motile Cells	23.6	21.3	23.2	23.2

[a]Different from control at $p<0.01$.
[b]Different from control at $p<0.05$.

Table 9. Blood Hematology Values[a] of Male Rats Following 28 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	11.0 ± 0.8	11.3 ± 1.0	12.1 ± 1.0	10.7 ± 0.9
RBC (10^6)	8.1 ± 0.2	7.9 ± 0.2	8.1 ± 0.2	7.5 ± 0.1
HGB (g/dL)	14.4 ± 0.2	14.1 ± 0.1	13.9 ± 0.2	13.1 ± 0.2
HCT (%)	45.5 ± 0.7	44.7 ± 0.4	44.4 ± 0.8	42.5 ± 0.7
MCV (fL)	56.2 ± 1.2	56.3 ± 0.7	54.8 ± 1.3	56.6 ± 0.6
MCH (pg)	17.8 ± 0.3	17.7 ± 0.2	17.2 ± 0.4	17.4 ± 0.2
MCHC (g/dL)	31.7 ± 0.3	31.5 ± 0.2	31.4 ± 0.3	30.9 ± 0.2
MetHb (%)	1.0 ± 0.1	1.0 ± 0.0	1.7 ± 0.1[b]	2.2 ± 0.2[b]
Platelets (10^3)	787.0 ± 54.2	724.7 ± 15.9	809.5 ± 40.6	814.8 ± 30.6
Neutrophils (%)	12.4 ± 2.6	10.2 ± 1.7	15.9 ± 1.3	13.3 ± 1.5
Lymphocytes (%)	81.5 ± 2.6	80.4 ± 1.7	77.6 ± 1.3	81.1 ± 1.5
Monocytes (%)	1.6 ± 0.1	1.7 ± 0.3	1.6 ± 0.3	1.5 ± 0.2
Eosinophils (%)	1.0 ± 0.2	1.0 ± 0.1	0.8 ± 0.2	0.9 ± 0.1
Basophils (%)	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	0.6 ± 0.1

[a]Mean ± SEM, N=6.
[b]Significantly different from control at $p<0.01$.

Table 10. Blood Hematology Values[a] of Male Rats Following 90 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	11.2 ± 0.3	10.2 ± 0.4	11.6 ± 0.8	12.1 ± 0.8
RBC (10^6)	8.7 ± 0.1	7.9 ± 0.6	8.0 ± 0.4	8.1 ± 0.3
HGB (g/dL)	15.1 ± 0.3	13.8 ± 0.7	13.8 ± 0.7	14.3 ± 0.4
HCT (%)	46.2 ± 1.1	41.4 ± 3.0	43.1 ± 2.3	44.9 ± 1.2
MCV (fL)	53.3 ± 1.1	52.3 ± 0.4	54.0 ± 0.8	55.3 ± 0.9
MCH (pg)	17.4 ± 0.3	17.6 ± 0.5	17.3 ± 0.2	17.6 ± 0.3
MCHC (g/dL)	32.6 ± 0.2	33.7 ± 1.0	33.1 ± 0.2	31.8 ± 0.2
MetHb (%)	1.1 ± 0.0	1.2 ± 0.1	1.7 ± 0.1[b]	2.3 ± 0.1[b]
Platelets (10^3)	528.7 ± 62.6	515.5 ± 44.1	655.3 ± 37.6	616.5 ± 34.9
Neutrophils (%)	14.2 ± 1.9	14.2 ± 1.3	14.8 ± 1.2	13.5 ± 0.8
Lymphocytes (%)	75.9 ± 1.7	75.3 ± 1.2	76.8 ± 1.5	77.1 ± 1.4
Monocytes (%)	2.7 ± 0.2	3.1 ± 0.2	2.5 ± 0.2	2.4 ± 0.3
Eosinophils (%)	4.2 ± 1.4	4.4 ± 0.6	3.4 ± 0.8	4.3 ± 0.6
Basophils (%)	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.0	0.8 ± 0.1

[a]Mean ± SEM, N=6.
[b]Significantly different from control at $p<0.01$.

Table 11. Blood Hematology Values[a] of Female Rats Following 90 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	7.2 ± 0.5	7.8 ± 0.6	8.4 ± 0.4	9.3 ± 0.9
RBC (10^6)	7.7 ± 0.2	7.5 ± 0.2	7.5 ± 0.1	4.5 ± 0.2
HGB (g/dL)	14.2 ± 0.2	13.6 ± 0.3	13.4 ± 0.1	12.7 ± 0.2[b]
HCT (%)	43.2 ± 1.1	41.5 ± 1.3	41.2 ± 0.7	39.7 ± 0.6
MCV (fL)	55.8 ± 0.6	55.1 ± 0.6	55.2 ± 0.7	55.6 ± 0.6
MCH (pg)	18.5 ± 0.5	18.1 ± 0.4	18.0 ± 0.3	17.8 ± 0.2
MCHC (g/dL)	33.2 ± 1.0	33.0 ± 0.8	32.6 ± 0.3	32.0 ± 0.3
MetHb (%)	1.3 ± 0.0	1.4 ± 0.0	1.8 ± 0.1[b]	2.4 ± 0.1[b]
Platelets (10^3)	751.2 ± 32.9	747.1 ± 40.0	840.9 ± 27.2	788.5 ± 16.5
Neutrophils (%)	14.0 ± 1.3	12.3 ± 0.9	10.3 ± 0.7[c]	9.3 ± 0.9[b]
Lymphocytes (%)	79.6 ± 1.2	81.6 ± 0.8	83.2 ± 0.8[c]	84.6 ± 1.1[b]
Monocytes (%)	2.4 ± 0.2	2.3 ± 0.2	2.5 ± 0.2	2.3 ± 0.2
Eosinophils (%)	1.2 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	1.0 ± 0.1
Basophils (%)	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.6 ± 0.1

[a]Mean ± SEM, N=12.
[b]Significantly different from control at $p<0.01$.
[c]Significantly different from control at $p<0.05$.

Table 12. Blood Hematology Values[a] of Male Rats Treated with TNB,60-Days Posttreatment

	CONTROL	LOW	MEDIUM	HIGH
WBC (10^3)	12.7 ± 0.8	13.0 ± 1.2	12.3 ± 0.5	12.0 ± 0.8
RBC (10^6)	9.2 ± 0.1	9.0 ± 0.1	8.9 ± 0.1	8.9 ± 0.1
HGB (g/dL)	15.3 ± 0.3	15.0 ± 0.4	15.3 ± 0.1	15.5 ± 0.1
HCT (%)	48.1 ± 0.7	46.3 ± 1.0	47.7 ± 0.4	47.8 ± 0.4
MCV (fL)	52.0 ± 0.8	51.7 ± 0.9	53.4 ± 0.5	53.9 ± 0.5
MCH (pg)	16.6 ± 0.3	16.7 ± 0.4	7.1 ± 0.1	17.4 ± 0.1
MCHC (g/dL)	31.9 ± 0.2	32.3 ± 0.2	2.0 ± 0.3	32.3 ± 0.2
MetHb (%)	1.2 ± 0.0	1.2 ± 0.0	1.4 ± 0.1	1.3 ± 0.0
Platelets (10^3)	851.3 ± 50.1	842.0 ± 36.8	890.3 ± 48.1	801.7 ± 39.1
Neutrophils (%)	13.1 ± 0.9	12.0 ± 0.7	12.3 ± 1.0	15.4 ± 3.0
Lymphocytes (%)	79.8 ± 1.2	81.1 ± 1.3	79.9 ± 1.1	78.4 ± 3.0
Monocytes (%)	2.4 ± 0.4	2.0 ± 0.3	2.6 ± 0.3	2.0 ± 0.2
Eosinophils (%)	1.3 ± 0.2	1.0 ± 0.1	1.3 ± 0.2	0.9 ± 0.2
Basophils (%)	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.9 ± 0.1

[a]Mean ± SEM, N=6.

Table 13. Mean Values[a] of Serum Chemistry Parameters for Male Rats Following 28 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/dL)	18.1 ± 1.2	17.8 ± 1.4	17.8 ± 0.8	16.7 ± 1.4
Creatinine (mg/dL)	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Chloride (mmol/L)	100.6 ± 0.9	99.3 ± 1.2	98.2 ± 1.0	96.4 ± 0.8
Calcium (mg/dL)	10.3 ± 0.1	11.1 ± 0.1	11.0 ± 0.4	11.4 ± 0.1[b]
Phosphorus (mg/dL)	10.7 ± 0.7	11.2 ± 0.6	12.5 ± 0.5	12.2 ± 0.6
Total Protein (g/dL)	6.4 ± 0.1	6.3 ± 0.1	6.5 ± 0.1	6.3 ± 0.1
AST (IU/L)	129.8 ± 13.5	105.3 ± 3.2	115.7 ± 12.3	119.8 ± 11.7
ALT (IU/L)	48.3 ± 4.0	44.3 ± 1.8	49.2 ± 4.3	51.2 ± 7.6
Alkaline phosphatase (IU/L)	174.8 ± 11.2	122.0 ± 5.2[b]	152.0 ± 12.6	166.0 ± 12.8
Albumin (g/dL)	3.7 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.4 ± 0.1
Glucose (mg/dL)	181.2 ± 16.0	157.3 ± 15.1	164.8 ± 17.3	162.2 ± 12.1
Sodium (mmol/L)	152.5 ± 0.5	151.0 ± 0.9	151.0 ± 0.7	150.0 ± 0.5
Triglycerides (mg/dL)	72.7 ± 11.6	67.5 ± 18.1	52.2 ± 9.7	40.0 ± 6.3
Magnesium (mg/dL)	2.9 ± 0.2	2.9 ± 0.1	3.1 ± 0.2	3.3 ± 0.1
Potassium (mmol/L)	5.7 ± 0.2	6.0 ± 0.2	7.0 ± 0.5	6.1 ± 0.5
Cholesterol (mg/dL)	55.8 ± 4.4	52.8 ± 6.1	48.0 ± 3.0	49.2 ± 2.14
Total Bilirubin (mg/dL)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0

[a]Mean ± SEM, N=6.
[b]Significantly different than control at $p<0.01$.

Table 14. Mean Values[a] of Serum Chemistry Parameters for Male Rats Following 90 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/dL)	15.9 ± 1.5	13.8 ± 0.9	16.1 ± 1.2	15.6 ± 1.2
Creatinine (mg/dL)	0.7 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	0.7 ± 0.0
Chloride (mmol/L)	96.0 ± 0.6	97.5 ± 0.7	98.0 ± 0.6	96.1 ± 1.2
Calcium (mg/dL)	11.6 ± 0.2	11.6 ± 0.1	11.6 ± 0.2	11.4 ± 0.2
Phosphorus (mg/dL)	10.6 ± 0.6	10.3 ± 0.8	11.1 ± 1.0	11.4 ± 0.7
Total Protein (g/dL)	6.6 ± 0.1	6.4 ± 0.2	6.5 ± 0.1	6.6 ± 0.1
AST (IU/L)	187.3 ± 16.1	171.8 ± 9.4	207.8 ± 36.9	159.2 ± 19.9
ALT (IU/L)	52.7 ± 4.0	48.0 ± 2.1	48.0 ± 4.7	44.7 ± 3.6
Alkaline phosphatase (IU/L)	111.5 ± 7.5	84.0 ± 7.2	89.5 ± 7.6	87.7 ± 6.4
Albumin (g/dL)	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.0	3.6 ± 0.1
Glucose (mg/dL)	204.0 ± 10.0	195.7 ± 16.7	191.3 ± 17.3	178.2 ± 7.6
Sodium (mmol/L)	149.2 ± 0.5	151.2 ± 0.4	150.2 ± 0.4	151.5 ± 0.4
Triglycerides (mg/dL)	73.8 ± 9.4	70.5 ± 25.6	53.3 ± 9.9	70.3 ± 15.5
Magnesium (mg/dL)	3.2 ± 0.2	3.1 ± 0.1	3.3 ± 0.3	3.8 ± 0.3
Potassium (mmol/L)	5.9 ± 0.3	5.5 ± 0.4	6.0 ± 0.4	5.7 ± 0.3
Cholesterol (mg/dL)	63.0 ± 4.4	48.7 ± 2.0	62.0 ± 7.9	52.3 ± 4.7
Total Bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0

[a]Mean ± SEM, N=6.

Table 15. Mean Values[a] of Serum Chemistry Parameters for Male Rats Treated with TNB, 60-Days Posttreatment

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/dL)	18.9 ± 1.5	18.2 ± 1.1	18.7 ± 1.0	18.1 ± 0.6
Creatinine (mg/dL)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Chloride (mmol/L)	98.0 ± 1.0	99.2 ± 0.8	100.2 ± 0.9	99.3 ± 0.8
Calcium (mg/dL)	12.1 ± 0.1	11.8 ± 0.1	11.9 ± 0.1	12.2 ± 0.1
Phosphorus (mg/dL)	11.4 ± 1.1	10.8 ± 0.6	9.5 ± 0.4	12.4 ± 0.9
Total Protein (g/dL)	6.9 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.8 ± 0.1
AST (IU/L)	100.8 ± 5.2	86.0 ± 5.0	92.0 ± 5.5	105.0 ± 4.4
ALT (IU/L)	52.4 ± 5.0	46.5 ± 2.6	52.0 ± 3.5	55.6 ± 4.0
Alkaline phosphatase (IU/L)	133.4 ± 21.9	114.2 ± 17.4	112.6 ± 15.3	134.8 ± 15.7
Albumin (g/dL)	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	4.1 ± 0.1
Glucose (mg/dL)	201.8 ± 23.9	200.3 ± 24.7	192.4 ± 10.4	187.0 ± 8.1
Sodium (mmol/L)	143.8 ± 0.7	143.3 ± 0.6	144.6 ± 0.7	144.2 ± 0.4
Triglycerides (mg/dL)	115.8 ± 13.9	143.7 ± 19.7	173.0 ± 16.8	85.4 ± 10.0
Magnesium (mg/dL)	4.1 ± 0.2	3.9 ± 0.3	3.4 ± 0.2	4.3 ± 0.3
Potassium (mmol/L)	6.5 ± 0.5	7.0 ± 0.4	7.0 ± 0.4	6.4 ± 0.2
Cholesterol (mg/dL)	74.2 ± 2.7	61.2 ± 3.6	73.0 ± 4.5	63.4 ± 5.9
Total Bilirubin (mg/dL)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0

[a]Mean ± SEM, N=6.

Table 16. Mean Values[a] of Serum Chemistry Parameters for Female Rats Following 90 Days of Treatment with TNB

	CONTROL	LOW	MEDIUM	HIGH
BUN (mg/dL)	17.0 ± 1.0	18.2 ± 0.9	17.7 ± 0.9	17.9 ± 1.0
Creatinine (mg/dL)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0
Chloride (mmol/L)	98.7 ± 0.9	99.8 ± 0.6	98.3 ± 0.7	99.1 ± 0.7
Calcium (mg/dL)	11.6 ± 0.2	12.0 ± 0.1	12.0 ± 0.1	12.1 ± 0.1
Phosphorus (mg/dL)	8.9 ± 0.3	8.6 ± 0.3	8.5 ± 0.3	9.4 ± 0.4
Total Protein (g/dL)	7.5 ± 0.1	7.7 ± 0.2	7.7 ± 0.1	7.8 ± 0.1
AST (IU/L)	203.8 ± 19.5	184.8 ± 27.0	190.3 ± 19.3	182.8 ± 17.4
ALT (IU/L)	54.2 ± 2.5	61.1 ± 5.7	47.8 ± 2.5	49.4 ± 2.9
Alkaline phosphatase (IU/L)	101.3 ± 7.9	99.2 ± 14.2	83.3 ± 8.5	100.8 ± 9.3
Albumin (g/dL)	4.5 ± 0.1	4.6 ± 0.2	4.6 ± 0.1	4.6 ± 0.1
Glucose (mg/dL)	231.3 ± 7.6	253.9 ± 15.3	224.7 ± 9.3	195.4 ± 10.1
Sodium (mmol/L)	150.8 ± 0.6	150.4 ± 0.4	149.3 ± 0.6	149.8 ± 0.9
Triglycerides (mg/dL)	89.1 ± 8.7	99.3 ± 18.6	78.3 ± 9.3	77.6 ± 5.9
Magnesium (mg/dL)	3.7 ± 0.1	3.5 ± 0.1	3.4 ± 0.1	3.6 ± 0.1
Potassium (mmol/L)	6.0 ± 0.2	6.6 ± 0.3	6.2 ± 0.2	6.3 ± 0.2
Cholesterol (mg/dL)	78.8 ± 4.7	74.7 ± 5.1	90.8 ± 4.5	101.3 ± 6.5[b]
Total Bilirubin (mg/dL)	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

[a]Mean ± SEM, N=12.
[b]Significantly different than control at $p<0.05$.

Table 17. Incidence Summary of Selected Microscopic Lesions of Male Rats Following 28 Days of Treatment with TNB

Organ/Lesion	Control	Low	Medium	High
Kidney (N)	6	6	6	6
Lymphoid infiltrates	100	67	17[c]	50
(severity)[a]	1.0	0.7	0.2[c]	0.8
Hyaline droplets	100	100	100	100
(severity)	2.8	2.7	2.3	2.2
Spleen (N)	6	6	6	6
Hemosiderosis	0	0	50[c]	100[b]
(severity)	0.0	0.0	0.5[c]	1.0[b]
Testes (N)	6	6	6	6
Tubular degeneration	17	0	0	0
(severity)	0.3	0.0	0.0	0.0
Epididymis (N)	6	6	6	6
Atypical sperm[d]	0	0	0	0

[a]Mean grades of severity based on 0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked; 5 = Severe.
[b]Statistically different from control at $p<0.01$.
[c]Statistically different from control at $p<0.05$.
[d]Signifys lesion present only, severity not graded.

Table 18. Incidence Summary of Selected Microscopic Lesions of Male Rats Following 90 Days of Treatment with TNB

Organ/Lesion	Control	Low	Medium	High
Kidney (N)	6	6	6	6
Lymphoid infiltrates	67	100	100	100
(severity)[a]	0.7	1.0	1.0	1.3[c]
Hyaline droplets	33	100[b]	100[b]	100[b]
(severity)	0.5	1.2	2.2[c]	3.0[c]
Spleen (N)	6	6	6	6
Hemosiderosis	100	100	100	100
(severity)	1.3	1.3	1.5	2.2[b]
Testes (N)	6	6	6	6
Tubular degeneration	0	0	0	50[c]
(severity)	0.0	0.0	0.0	1.0[b]
Epididymis (N)	6	6	6	6
Atypical sperm[d]	0	0	0	50[c]

[a]Mean grades of severity based on 0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked; 5 = Severe.
[b]Statistically different from control at $p<0.01$.
[c]Statistically different from control at $p<0.05$.
[d]Signifys lesion present only, severity not graded.

Table 19. Incidence Summary of Selected Microscopic Lesions of Female Rats Following 90 Days of Treatment with TNB

Organ/Lesion	Control	Low	Medium	High
Kidney (N)	12	12	12	12
Lymphoid infiltrates	42	58	17	58
(severity)[a]	0.4	0.6	0.2	0.8
Hyaline droplets	0	0	33	66[b]
(severity)	0.0	0.0	0.3	0.8[b]
Spleen (N)	12	12	12	12
Hemosiderosis	100	100	100	100
(severity)	1.2	1.3	2.4[b]	3.0[b]

[a]Mean grades of severity based on 0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked; 5 = Severe.
[b]Statistically different from control at $p<0.01$.

Table 20. Incidence Summary of Selected Microscopic Lesions of Male Rats Two Months Following Treatment with TNB

Organ/Lesion	Control	Low	Medium	High
Kidney (N)	6	6	6	6
Lymphoid infiltrates	100	100	83	100
(severity)[a]	1.3	1.0	0.8	1.3
Hyaline droplets	100	100	83	83
(severity)	1.0	1.0	0.8	0.8
Spleen (N)	6	6	6	6
Hemosiderosis	83	100	100	100
(severity)	1.2	1.5	1.8	1.8
Testes (N)	6	6	6	5
Tubular degeneration	0	0	0	40[c]
(severity)	0.0	0.0	0.0	0.6[c]
Epididymis (N)	6	6	6	5
Atypical sperm[d]	0	0	17	20

[a]Mean grades of severity based on 0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Marked; 5 = Severe.
[b]Statistically different from control at $p<0.01$.
[c]Statistically different from control at $p<0.05$.
[d]Signifys lesion present only, severity not graded.

Table 21. Litter Data for Rats Treated with TNB

	Control	Low	Medium	High
No. of Mated Pairs	12	12	12	12
No. of Copulated Pairs	12	12	12	11
No. of Dams with Pups Born	12	12	12	11
No. of Dams with Pups Alive	12	12	12	11
Fertility Index (%)	100.0	100.0	100.0	91.7
Gestation Index (%)[a]	100.0	100.0	100.0	100.0
Live Birth Index (%)[b]	97.5	100.0	97.8	98.1
4-Day Survival Index (%)	98.7	100.0	100.0	98.1
7-Day Survival Index (%)	100.0	98.9	100.0	100.0
14-Day Survival Index (%)	100.0	100.0	100.0	100.0
21-Day Survival Index (%)	100.0	100.0	100.0	100.0
Lactation Index (%)[c]	100.0	98.9	100.0	100.0

[a] $\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}}$

[b] $\frac{\text{Number of live pups at birth}}{\text{Total number of pups born}}$

[c] $\frac{\text{Number of pups surviving 21 days}}{\text{Number of pups surviving 4 days}}$

SECTION 5

REFERENCES

Barcikowski, R.S., ed. 1983. *Computer Packages and Research Design.* Lanham, MD: University Press of America.

Barnhart, R.R. 1981. Rubber Compounding. In: *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd Ed., Vol. 20. (A. Stannden, Ed.) John Wiley and Sons, New York, NY. pp. 393-468.

Budavari, S., M.J. O'Neil, and A. Smith, Eds. 1989. In: *The Merck Index.* Merck & Co., Inc., Rahway, NJ. pp. 1530.

Cody, T.E., S. Witherup, L. Hastings, K. Stemmer, and R.T. Christian. 1981. 1,3-Dinitrobenzene: Toxic effect *in vivo* and *in vitro*. *J. Toxicol. Environ. Health* **7**(5):829-847.

Fedoroff, B.T., O.E. Sheffield, E.F. Reese, and G.D. Clift, Eds. 1962. In: *Encyclopedia of Explosives and Related Items.* PATR 2700, Vol. 2. Picatinny Arsenal, Dover, NJ. pp. B48-B49.

Fitzgerald, C.B., N. DiGiulio, L.S. Desai, and G. Reddy. 1992. Acute toxicological evaluation of 1,3,5-Trinitrobenzene. *Acute Toxicity Data* **1**(3):169-170.

Furedi, E.M., B.S. Levine, D.E. Gordon, V.S. Rac, and P.M. Lish. 1984a. *Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the Fischer 344 rat).* Final Report - Phase III, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.

Furedi, E.M., B.S. Levine, J.W. Sagartz, V.S. Rac, and P.M. Lish. 1984b. *Determination of the chronic mammalian toxicologic effects of TNT (Twenty-four month chronic toxicity/carcinogenicity study of trinitrotoluene (TNT) in the B6C3F$_1$ hybrid mouse).* Final Report - Phase IV, Vol. 1. IIT Research Institute, Project No. L6116, Study No. 11, Chicago, IL. DAMD17-79-C-9120. AD-A168 754.

Hathaway, J.A. 1977. Trinitrotoluene: A review of reported dose-related effects providing documentation for a workplace standard. *J. Occup. Med.* **19**(5):341-345.

Kinkead, E.R., S.K. Bunger, E.C. Kimmel, C.D. Flemming, H.G. Wall, and J.H. Grabau. 1991. Effects of a 13-Week Chloropentafluorobenzene Inhalation Exposure of Fischer 344 Rats and B6C3F1 Mice. *Tox. and Ind. Health* **7**(4):309-318.

Kinkead, E.R., R.E. Wolfe, S.A. Salins, C.D. Flemming, D.J. Caldwell, C.R. Miller, and J.R. Latendresse. 1994. *Range-Finding Study for a Reproductive Assessment of 1,3,5-Trinitrobenzene Administered in the Diet of Sprague-Dawley Rats.* AL-TR-1994-072/WRAIR-TR-1994-0006. Wright-Patterson Air Force Base, OH: Armstrong Laboratory and Walter Reed Army Institute of Research.

Levine, B.S., J.H. Rust, J.M. Burns, and P.M. Lish. 1983. *Determination of the chronic mammalian toxicological effects of TNT (Twenty-six week subchronic oral toxicity study of trinitrotoluene (TNT) in the beagle dog).* Phase II, Final Report, IIT Research Institute, Report No. L6116, Study No. 5, Chicago, IL. DAMD 17-79-C-9120, AD-A157 082.

Levine, B.S., E.M. Furedi, D.E. Gordon, P.M. Lish, and J.J. Barkely. 1984. Subchronic toxicity of trinitrotoluene in Fischer 344 rats. *Toxicology* **32**:253-265.

Linder, R.E., R.A. Hess, and L.F. Strader. 1986. Testicular toxicity and infertility in male rats treated with 1,3-dinitrobenzene. *J. Tox. and Environ. Health* **19**:477-489.

Linder, R.E., L.F. Strader, R.R. Barbee, G.L. Rehnberg, and S.D. Perreault. 1990. Reproductive toxicity of a single dose of 1,3-dinitrobenzene in two ages of young adult male rats. *Fundam. Appl. Tox.* **14**:284-298.

Morton, A.R., M.V. Ranadive, and J.A. Hathaway. 1976. Biological effects of trinitrotoluene from exposure below the threshold limit value. *Am. Ind. Hyg. Assoc. J.* **37**(1):56-60.

Philbert, M.A., A.J. Gray, and T.A. Connors. 1987. Preliminary investigations into the involvement of the intestinal microfluora in CNS toxicity induced by 1,3-dinitrobenzene in male F-344 rats. *Tox Letters* **38**:307-314.

Rosner, B. 1990. *Fundamentals of Biostatistics.* Boston, MA.: PWS-Kent Publishing Co.

Ryon, M.G., B.C. Pal, S.S. Talmage, and R.H. Ross. 1984. *Database assessment of the health and environmental effects of munition production waste products.* Final Report. ORNL-6018. (NTIS DE84-016512). Oak Ridge National Laboratory, Oak Ridge, TN.

Sokal, R.R. and F.J. Rohlf. 1981. *Biometry,* Second Edition, W.W. Freeman and Company, New York, NY.

Stewart, A., L.T. Witts, G. Higgins, et al. 1945. Some early effects of exposure to trinitrotoluene. *Br. J. Ind. Med.* **2**:74-82. (Cited in Hathaway, 1977)

Toth, G.P., S.R. Wang, H. McCarthy, D.R. Tocco, and M.K. Smith. 1992. Effects of three male reproductive toxicants on rat cauda epididymal sperm motion. *Reproductive Toxicology* **6**:507-515.

U.S. EPA. 1989. *Health and Environmental Effects Profile for 1,3,5-Trinitrobenzene.* Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH, ECAO-CIN-G071.

APPENDIX A.

DIET PREPARATION AND ANALYSIS

DIET

1,3,5-Trinitrobenzene powder (CAS# 99-35-4) was supplied by the U.S. Army Biomedical Research and Development Laboratory. Analysis by HPLC revealed no detectable impurities. Certified powdered Purina Laboratory Chow 5002 was purchased (Ralston Purina Co., St. Louis, MO) and stored at 4 °C until used. TNB diets were prepared as needed. First, 1.2 g of TNB was added to 50 g of powdered diet in a mortar and thoroughly ground with a pestle. Afterwards, 200 g of the diet was added and mixed for 15 minutes, followed by 550 g and mixed for an additional 15 minutes. Finally, the remaining diet (700 g) was added and mixed for 30 minutes in a mechanical mixer (Kitchen Aid, St. Joseph, MI) for uniform distribution of TNB in the diet. This was verified by determining the TNB concentration in the diet, taken from each of the 1 kg mixtures, by quantitative analysis done by HPLC. The premixed diet (0.8 g/kg) was further diluted with fresh powdered diet to obtain the desired TNB concentration in the lower dose groups. The diet feeders were changed twice a week.

DIET ANALYSIS

Analyses of the TNB-feed mixtures were carried out on acetone extracts of the mixtures, utilizing a Waters 600E chromatography system (Waters, Milford, MA), equipped with a 490E programmable multiwavelength detector, operating at 254 nm. The entire chromatography system was interfaced with a Berthold HPLC computer program, Version 1.65 (Berthold, Nashua, NH). The TNB was eluted from a Zorbax C-8 column (9.4 mm x 25 cm) (MAC-DOD Analytical, Chadds Ford, PA) with a water-methanol gradient, at a flow rate of 3 mL/min. Working standards were prepared in Burdick and Jackson HPLC grade high purity methanol (Baxter, Obetz, OH).

APPENDIX B.

MEAN BODY WEIGHTS[a] OF MALE RATS TREATED WITH TNB FOR 90 DAYS

DAY	CONTROL	LOW	MEDIUM	HIGH
0	355 ± 4.0	355 ± 4.0	355 ± 3.9	355 ± 4.0
7	392 ± 5.7	391 ± 4.9	385 ± 4.7	375 ± 4.9
14	419 ± 7.1	421 ± 5.3	411 ± 5.7	399 ± 5.3
21	442 ± 7.3	439 ± 5.7	429 ± 6.7	415 ± 6.1[b]
28	466 ± 8.3	477 ± 10.4	449 ± 6.7	433 ± 6.0[b]
35	488 ± 10.1	482 ± 7.4	466 ± 8.8	452 ± 8.4[b]
42	510 ± 11.1	501 ± 8.6	487 ± 9.4	472 ± 9.2[b]
49	527 ± 11.0	515 ± 8.9	506 ± 9.7	486 ± 9.2[b]
56	542 ± 11.3	530 ± 8.8	521 ± 9.1	499 ± 9.9[b]
63	558 ± 11.8	543 ± 9.3	531 ± 9.6	509 ± 10.3[c]
70	572 ± 12.5	554 ± 9.6	543 ± 9.7	519 ± 9.4[c]
77	581 ± 12.9	564 ± 10.6	551 ± 9.6	524 ± 10.1[c]
84	586 ± 13.4	571 ± 11.2	557 ± 9.9	530 ± 10.1[c]
91	595 ± 12.7	579 ± 12.7	560 ± 9.9	535 ± 9.3[c]
105	613 ± 17.4	625 ± 17.4	608 ± 14.5	565 ± 18.1
120	645 ± 24.4	637 ± 19.1	629 ± 18.8	596 ± 17.5
133	665 ± 25.5	663 ± 25.1	654 ± 19.1	617 ± 18.2
147	675 ± 28.9	676 ± 24.9	669 ± 22.7	629 ± 19.6

[a]Mean ± SEM; N=18 on Days 0 through 28, N=12 on Days 35 through 91, N=6 on Days 105 through 147.
[b]Significantly different from control at $p<0.05$.
[c]Significantly different from control at $p<0.01$.

APPENDIX C.

MEAN BODY WEIGHTS[a] OF FEMALE RATS TREATED WITH TNB

DAY	CONTROL	LOW	MEDIUM	HIGH
PM0	243 ± 3.4	243 ± 4.3	243 ± 3.6	242 ± 3.4
PM7	252 ± 4.6	255 ± 5.0	250 ± 4.9	246 ± 3.7
PM14	264 ± 5.8	268 ± 6.8	261 ± 5.2	254 ± 5.2
G0	253 ± 5.2	274 ± 7.1	261 ± 4.8	254 ± 5.2
G7	305 ± 5.7	316 ± 8.3	305 ± 5.8	293 ± 5.4
G14	347 ± 4.8	355 ± 9.1	344 ± 6.5	327 ± 5.0
G20	420 ± 6.8	432 ± 12.0	420 ± 7.8	398 ± 6.6
L0	335 ± 5.9	341 ± 8.6	329 ± 5.4	300 ± 7.1[b]
L4	347 ± 7.7	355 ± 9.0	346 ± 5.2	325 ± 7.4
L7	352 ± 6.6	358 ± 8.8	351 ± 5.8	329 ± 6.1
L14	367 ± 6.5	376 ± 9.4	361 ± 4.9	345 ± 7.8
L21	343 ± 6.5	357 ± 7.5	347 ± 5.1	331 ± 8.0
PW1	328 ± 6.5	337 ± 9.6	332 ± 7.8	308 ± 5.8
PW2	329 ± 6.6	346 ± 10.1	325 ± 8.5	308 ± 6.5
PW3	332 ± 7.9	348 ± 11.8	327 ± 8.6	309 ± 6.7

[a]Mean ± SEM; N=12.
[b]Different than control at $p<0.01$.

PM: Premating
G: Gestation
L: Lactation
PW: Postweaning

APPENDIX D.

MEAN BODY WEIGHTS[a] OF MALE AND FEMALE RAT PUPS

DAY	CONTROL	LOW	MEDIUM	HIGH
Day 1	7.37 ± 0.07	6.72 ± 0.05[b]	6.89 ± 0.07[b]	6.90 ± 0.08[b]
N	154	175	176	155
Day 4	11.30 ± 0.13	10.02 ± 0.12[b]	10.27 ± 0.12[b]	9.90 ± 0.12[b]
N	154	174	174	155
Day 7	18.80 ± 0.22	16.73 ± 0.30[b]	17.56 ± 0.27[b]	16.36 ± 0.25[b]
N	96	91	92	88
Day 14	36.31 ± 0.32	34.74 ± 0.53	36.47 ± 0.43	30.63 ± 0.65[b]
N	96	91	92	88
Day 21	59.70 ± 0.55	55.13 ± 0.80[b]	55.91 ± 0.80[b]	51.60 ± 0.65[b]
N	96	91	92	88

[a]Mean ± SEM.
[b]Significantly different than control at $p<0.01$.

U. S. Government Printing Office 1995 650-075/00068